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APPLICATION

FOR

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Title: **TISSUE-SPECIFIC EXOGENOUS OPTICAL AGENTS**

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TISSUE-SPECIFIC EXOGENOUS OPTICAL AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Divisional of U.S. Patent Application Serial No. 09/688,949, filed on October 16, 2000, now pending, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

This invention relates to novel optical probes for use in physiological function monitoring, particularly indole and benzoindole compounds.

BACKGROUND OF THE INVENTION

Dynamic monitoring of physiological functions of patients at the bedside is highly desirable in order to minimize the risk of acute renal failure brought about by various clinical, physiological, and pathological conditions (C.A. Rabito, L.S.T. Fang, and A.C. Waltman, Renal function in patients at risk with contrast material-induced acute renal failure: Noninvasive real-time monitoring, *Radiology* 1993, 186, 851-854; N.L. Tilney, and J.M. Lazarus, Acute renal failure in surgical patients: Causes, clinical patterns, and care, *Surgical Clinics of North America*, 1983, 63, 357-377; B.E. VanZe, W.E. Hoy,

and J.R. Jaenike, Renal injury associated with intravenous pyelography in non-diabetic and diabetic patients, *Annals of Internal Medicine*, 1978, 89, 51-54; S. Lundqvist, G. Edbom, S. Groth, U. Stendahl, and S.-O. Hietala, Iohexol clearance for renal function measurement in gynecologic cancer patients, *Acta Radiologica*, 1996, 37, 582-586; P. Guesry, L. Kaufman, S. Orlof, J.A. Nelson, S. Swann, and M. Holliday, Measurement of glomerular filtration rate by fluorescent excitation of non-radioactive meglumine iothalamate, *Clinical Nephrology*, 1975, 3, 134-138). This monitoring is particularly important in the case of critically ill or injured patients because a large percentage of these patients face the risk of multiple organ failure (MOF), resulting in death (C.C. Baker et al., Epidemiology of Trauma Deaths, *American Journal of Surgery*, 1980, 144-150; R.G. Lobenhofer et al., Treatment Results of Patients with Multiple Trauma: An Analysis of 3406 Cases Treated Between 1972 and 1991 at a German Level I Trauma Center, *Journal of Trauma*, 1995, 38, 70-77). MOF is a sequential failure of lung, liver, and kidneys, and is incited by one or more severe causes such as acute lung injury (ALI), adult respiratory distress syndrome (ARDS), hypermetabolism, hypotension, persistent inflammatory focus, or sepsis syndrome. The common histological features of hypotension and shock leading to MOF include tissue necrosis, vascular congestion, interstitial and cellular edema, hemorrhage, and microthrombi. These changes affect the lung, liver, kidneys, intestine, adrenal glands, brain, and pancreas, in descending order of frequency (J. Coalson, Pathology of Sepsis, Septic Shock, and Multiple Organ Failure. In New Horizons: Multiple Organ Failure, D.J. Bihari and F.B. Cerra (Eds). *Society of Critical Care*

Medicine, Fullerton, CA, 1986, pp. 27-59). The transition from early stages of trauma to clinical MOF is marked by the extent of liver and renal failure and a change in mortality risk from about 30% to about 50% (F.B. Cerra, Multiple Organ Failure Syndrome. In New Horizons: Multiple Organ Failure, D.J. Bihari and F.B. Cerra (Eds). *Society of Critical Care Medicine*, Fullerton, CA, 1989, pp. 1-24).

Serum creatinine measured at frequent intervals by clinical laboratories is currently the most common way of assessing renal function and following the dynamic changes in renal function which occur in critically ill patients (P.D. Dollan, E.L. Alpen, and G.B. Theil, A clinical appraisal of the plasma concentration and endogenous clearance of creatinine, *American Journal of Medicine*, 1962, 32, 65-79; J.B. Henry (Ed). Clinical Diagnosis and Management by Laboratory Methods, 17th Edition, W.B. Saunders, Philadelphia, PA, 1984); C.E. Speicher, The right test: A physician's guide to laboratory medicine, W.B. Saunders, Philadelphia, PA, 1989). These values are frequently misleading, since age, state of hydration, renal perfusion, muscle mass, dietary intake, and many other clinical and anthropometric variables affect the value. In addition, a single value returned several hours after sampling is difficult to correlate with other important physiologic events such as blood pressure, cardiac output, state of hydration and other specific clinical events (e.g., hemorrhage, bacteremia, ventilator settings and others). An approximation of glomerular filtration rate can be made via a 24-hour urine collection, but this requires 24 hours to collect the sample, several more hours to analyze the sample, and a meticulous bedside collection technique. New

or repeat data are equally cumbersome to obtain. Occasionally, changes in serum creatinine must be further adjusted based on the values for urinary electrolytes, osmolality, and derived calculations such as the "renal failure index" or the "fractional excretion of sodium." These require additional samples of serum collected contemporaneously with urine samples and, after a delay, precise calculations. Frequently, dosing of medication is adjusted for renal function and thus can be equally as inaccurate, equally delayed, and as difficult to reassess as the values upon which they are based. Finally, clinical decisions in the critically ill population are often as important in their timing as they are in their accuracy.

Exogenous markers such as inulin, iohexol, ^{51}Cr-EDTA, Gd-DTPA, or ^{99m}Tc-DTPA have been reported to measure the glomerular filtration rate (GFR) (P.L. Choyke, H.A. Austin, and J.A. Frank, Hydrated clearance of gadolinium-DTPA as a measurement of glomerular filtration rate, *Kidney International*, 1992, 41, 1595-1598; M.F. Twedle, X. Zhang, M. Fernandez, P. Wedeking, A.D. Nunn, and H.W. Strauss, A noninvasive method for monitoring renal status at bedside, *Invest. Radiol.*, 1997, 32, 802-805; N. Lewis, R. Kerr, and C. Van Buren, Comparative evaluation of urographic contrast media, inulin, and ^{99m}Tc-DTPA clearance methods for determination of glomerular filtration rate in clinical transplantation, *Transplantation*, 1989, 48, 790-796). Other markers such as ^{123}I and ^{125}I labeled o-iodohippurate or ^{99m}Tc-MAG$_3$ are used to assess tubular secretion process (W.N. Tauxe, Tubular Function, in *Nuclear Medicine in Clinical Urology and Nephrology*, W.N. Tauxe and E.V. Dubovsky, Editors, pp. 77-105, Appleton Century Crofts,

East Norwalk, 1985; R. Muller-Suur, and C. Muller-Suur, Glomerular filtration and tubular secretion of MAG_3 in rat kidney, *Journal of Nuclear Medicine*, 1989, 30, 1986-1991). However, these markers have several undesirable properties such as the use of radioactivity or *ex-vivo* handling of blood and urine samples. Thus, in order to assess the status and to follow the progress of renal disease, there is a considerable interest in developing a simple, safe, accurate, and continuous method for determining renal function, preferably by non-radioactive procedures. Other organs and physiological functions that would benefit from real-time monitoring include the heart, the liver, and blood perfusion, especially in organ transplant patients.

Hydrophilic, anionic substances are generally recognized to be excreted by the kidneys (F. Roch-Ramel, K. Besseghir, and H. Murer, Renal excretion and tubular transport of organic anions and cations, *Handbook of Physiology, Section 8, Neurological Physiology, Vol. II,* E.E. Windhager, Editor, pp. 2189-2262, Oxford University Press, New York, 1992; D.L. Nosco, and J.A. Beaty-Nosco, Chemistry of technetium radiopharmaceuticals 1: Chemistry behind the development of technetium-99m compounds to determine kidney function, *Coordination Chemistry Reviews*, 1999, 184, 91-123). It is further recognized that drugs bearing sulfonate residues exhibit improved clearance through the kidneys (J. Baldas, J. Bonnyman, Preparation, HPLC studies and biological behavior of techentium-99m and 99mTcN0-radiopharmaceuticals based on quinoline type ligands, *Nucl. Med. Biol.*, 1999, 19, 491-496; L. Hansen, A. Taylor, L., L.G. Marzilli, Synthesis of the sulfonate and phosphonate derivatives of mercaptoacetyltriglycine. X-ray

crystal structure of Na_2[ReO(mercaptoacetylglycylglycylaminomethane-sulfonate)]·$3H_2O$, *Met.-Based Drugs*, 1994, 1, 31-39).

Assessment of renal function by continuously monitoring the blood clearance of exogenous optical markers, viz., fluorescein bioconjugates derived from anionic polypeptides, has been developed by us and by others (R.B. Dorshow, J.E. Bugaj, B.D. Burleigh, J.R. Duncan, M.A. Johnson, and W.B. Jones, Noninvasive fluorescence detection of hepatic and renal function, *Journal of Biomedical Optics*, 1998, 3, 340-345; M. Sohtell et al., FITC-Inulin as a Kidney Tubule Marker in the Rat, *Acta. Physiol. Scand.*, 1983, 119, 313-316, each of which is expressly incorporated herein by reference). The main drawback of high molecular weight polypeptides is that they are immunogenic. In addition, large polymers with narrow molecular weight distribution are difficult to prepare, especially in large quantities. Thus, there is a need in the art to develop low molecular weight compounds that absorb and/or emit light that can be used for assessing renal, hepatic, cardiac and other organ functions.

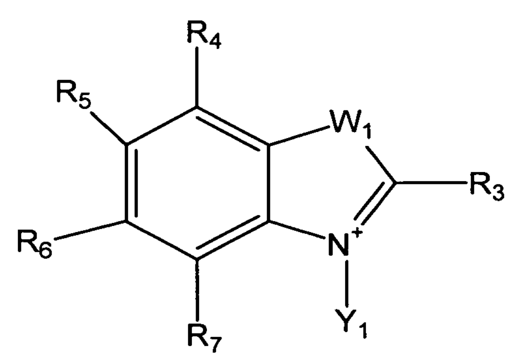
SUMMARY OF THE INVENTION

The present invention overcomes these difficulties by incorporating hydrophilic anionic or polyhydroxy residues in the form of sulfates, sulfonates, sulfamates and strategically positioned hydroxyl groups. Thus, the present invention is related to novel dyes containing multiple hydrophilic moieties and their use as diagnostic agents for assessing organ function.

The novel compositions of the present invention comprise dyes of Formulas 1 to 6 which are hydrophilic and absorb light in the visible and near infrared regions of the electromagnetic spectrum. The ease of modifying the clearance pathways of the dyes after *in vivo* administration permits their use for physiological monitoring. Thus, blood protein-binding compounds are useful for angiography and organ perfusion analysis, which is particularly useful in organ transplant and critical ill patients. Predominant kidney clearance of the dyes enables their use for dynamic renal function monitoring, and rapid liver uptake of the dyes from blood serves as a useful index for the evaluation of hepatic function.

As illustrated in Figures 1-7, these dyes are designed to inhibit aggregation in solution by preventing intramolecular and intermolecular induced hydrophobic interactions.

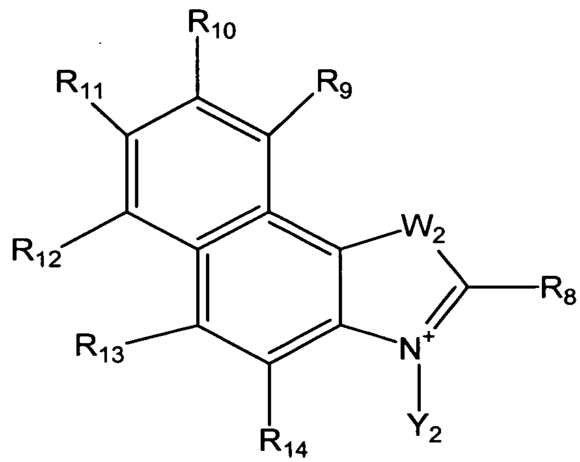
The present invention relates particularly to the novel compounds comprising indoles of the general Formula 1



Formula 1

wherein R_3, R_4, R_5, R_6, and R_7, and Y_1 are independently selected from the group consisting of -H, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, C1-C20 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, saccharides, amino, C1-C10 aminoalkyl, cyano, nitro, halogen, hydrophilic peptides, arylpolysulfonates, C1-C10 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-(CH_2)_aCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCO(CH_2)_bSO_3T$, $-(CH_2)_aNHCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCSNH(CH_2)_bSO_3T$, $-(CH_2)_aOCONH(CH_2)_bSO_3T$, $-(CH_2)_aPO_3HT$, $-(CH_2)_aPO_3T_2$, $-(CH_2)_aOPO_3HT$, $-(CH_2)_aOPO_3T_2$, $-(CH_2)_aNHPO_3HT$, $-(CH_2)_aNHPO_3T_2$, $-(CH_2)_aCO_2(CH_2)_bPO_3HT$, $-(CH_2)_aCO_2(CH_2)_bPO_3T_2$, $-(CH_2)_aOCO(CH_2)_bPO_3HT$, $-(CH_2)_aOCO(CH_2)_bPO_3T_2$, $-(CH_2)_aCONH(CH_2)_bPO_3HT$, $-(CH_2)_aCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCO(CH_2)_bPO_3HT$, $-(CH_2)_aNHCO(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCONH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3T_2$, $-(CH_2)_aOCONH(CH_2)_bPO_3HT$, and $-(CH_2)_aOCONH(CH_2)_bPO_3T_2$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_1 is selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; a, b, d, f, h, i, and j independently vary from 1-10; c, e, g, and k independently vary from 1-100; R_a, R_b, R_c, and R_d are defined in the same manner as Y_1; T is either H or a negative charge.

The present invention also relates to the novel compounds comprising benzoindoles of general Formula 2

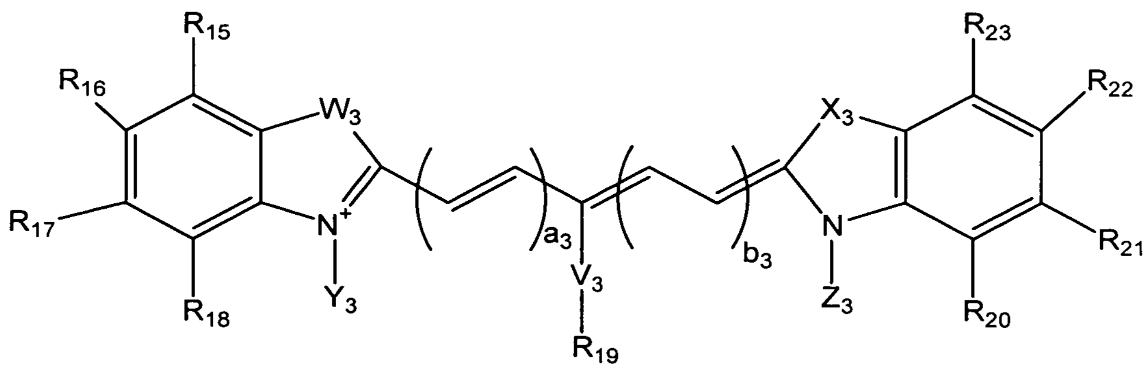


Formula 2

wherein R_8, R_9, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, and Y_2 are independently selected from the group consisting of -H, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, C1-C20 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, saccharides, amino, C1-C10 aminoalkyl, cyano, nitro, halogen, hydrophilic peptides, arylpolysulfonates, C1-C10 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-(CH_2)_aCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCO(CH_2)_bSO_3T$, $-(CH_2)_aNHCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCSNH(CH_2)_bSO_3T$, $-(CH_2)_aOCONH(CH_2)_bSO_3T$, $-(CH_2)_aPO_3HT$, $-(CH_2)_aPO_3T_2$, $-(CH_2)_aOPO_3HT$, $-(CH_2)_aOPO_3T_2$, $-(CH_2)_aNHPO_3HT$, $-(CH_2)_aNHPO_3T_2$, $-(CH_2)_aCO_2(CH_2)_bPO_3HT$, $-(CH_2)_aCO_2(CH_2)_bPO_3T_2$, $-(CH_2)_aOCO(CH_2)_bPO_3HT$, $-(CH_2)_aOCO(CH_2)_bPO_3T_2$, $-(CH_2)_aCONH(CH_2)_bPO_3HT$, $-(CH_2)_aCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCO(CH_2)_bPO_3HT$, $-(CH_2)_aNHCO(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCONH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCONH(CH_2)_bPO_3T_2$,

$-(CH_2)_aNHCSNH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3T_2$, $-(CH_2)_aOCONH(CH_2)_bPO_3HT$, and $-(CH_2)_aOCONH(CH_2)_bPO_3T_2$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_2 is selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; a, b, d, f, h, i, and j independently vary from 1-10; c, e, g, and k independently vary from 1-100; R_a, R_b, R_c, and R_d are defined in the same manner as Y_2; T is either H or a negative charge.

The present invention also relates to the novel composition comprising cyanine dyes of general Formula 3

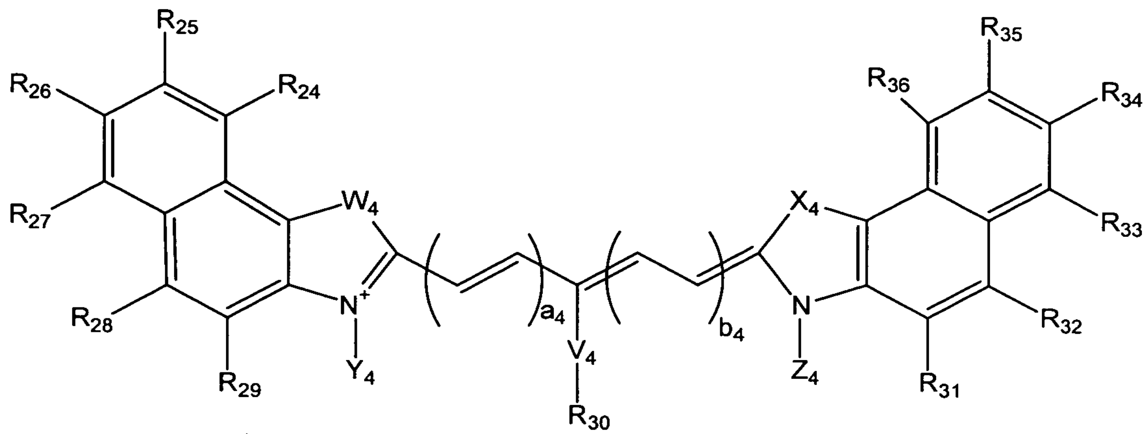


Formula 3

wherein R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, Y_3, and Z_3 are independently selected from the group consisting of -H, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, C1-C20 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, saccharides, amino, C1-C10 aminoalkyl, cyano, nitro, halogen, hydrophilic peptides, arylpolysulfonates, C1-C10 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-(CH_2)_aCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCO(CH_2)_bSO_3T$, $-(CH_2)_aNHCONH(CH_2)_bSO_3T$,

$-(CH_2)_aNHCSNH(CH_2)_bSO_3T$, $-(CH_2)_aOCONH(CH_2)_bSO_3T$, $-(CH_2)_aPO_3HT$, $-(CH_2)_aPO_3T_2$, $-(CH_2)_aOPO_3HT$, $-(CH_2)_aOPO_3T_2$, $-(CH_2)_aNHPO_3HT$, $-(CH_2)_aNHPO_3T_2$, $-(CH_2)_aCO_2(CH_2)_bPO_3HT$, $-(CH_2)_aCO_2(CH_2)_bPO_3T_2$, $-(CH_2)_aOCO(CH_2)_bPO_3HT$, $-(CH_2)_aOCO(CH_2)_bPO_3T_2$, $-(CH_2)_aCONH(CH_2)_bPO_3HT$, $-(CH_2)_aCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCO(CH_2)_bPO_3HT$, $-(CH_2)_aNHCO(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCONH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3T_2$, $-(CH_2)_aOCONH(CH_2)_bPO_3HT$, and $-(CH_2)_aOCONH(CH_2)_bPO_3T_2$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_3 and X_3 are selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; V_3 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and $-NR_a$; a, b, d, f, h, i, and j independently vary from 1-10; c, e, g, and k independently vary from 1-100; a_3 and b_3 vary from 0 to 5; R_a, R_b, R_c, and R_d are defined in the same manner as Y_3; T is either H or a negative charge.

The present invention further relates to the novel composition comprising cyanine dyes of general Formula 4

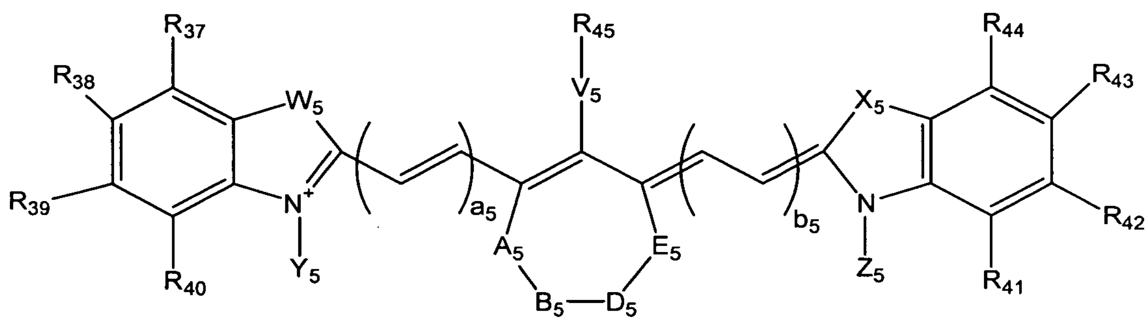


Formula 4

wherein R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, Y_4, and Z_4 are independently selected from the group consisting of -H, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, C1-C20 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, saccharides, amino, C1-C10 aminoalkyl, cyano, nitro, halogen, hydrophilic peptides, arylpolysulfonates, C1-C10 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-(CH_2)_aCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCO(CH_2)_bSO_3T$, $-(CH_2)_aNHCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCSNH(CH_2)_bSO_3T$, $-(CH_2)_aOCONH(CH_2)_bSO_3T$, $-(CH_2)_aPO_3HT$, $-(CH_2)_aPO_3T_2$, $-(CH_2)_aOPO_3HT$, $-(CH_2)_aOPO_3T_2$, $-(CH_2)_aNHPO_3HT$, $-(CH_2)_aNHPO_3T_2$, $-(CH_2)_aCO_2(CH_2)_bPO_3HT$, $-(CH_2)_aCO_2(CH_2)_bPO_3T_2$, $-(CH_2)_aOCO(CH_2)_bPO_3HT$, $-(CH_2)_aOCO(CH_2)_bPO_3T_2$, $-(CH_2)_aCONH(CH_2)_bPO_3HT$, $-(CH_2)_aCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCO(CH_2)_bPO_3HT$, $-(CH_2)_aNHCO(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCONH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3T_2$, $-(CH_2)_aOCONH(CH_2)_bPO_3HT$, and $-(CH_2)_aOCONH(CH_2)_bPO_3T_2$, $-CH_2(CH_2$-O-

$CH_2)_c$-CH_2-OH, -$(CH_2)_d$-CO_2T, -CH_2-$(CH_2$-O-$CH_2)_e$-CH_2-CO_2T, -$(CH_2)_f$-NH_2, -CH_2-$(CH_2$-O-$CH_2)_g$-CH_2-NH_2, -$(CH_2)_h$-N(R_a)-$(CH_2)_i$-CO_2T, and -$(CH_2)_j$-N(R_b)-CH_2-$(CH_2$-O-$CH_2)_k$-CH_2-CO_2T; W_4 and X_4 are selected from the group consisting of -CR_cR_d, -O-, -NR_c, -S-, and -Se; V_4 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and -NR_a; a_4 and b_4 vary from 0 to 5; a, b, d, f, h, i, and j independently vary from 1-10; c, e, g, and k independently vary from 1-100; R_a, R_b, R_c, and R_d are defined in the same manner as Y_4; T is either H or a negative charge.

The present invention also relates to the novel composition comprising cyanine dyes of general Formula 5

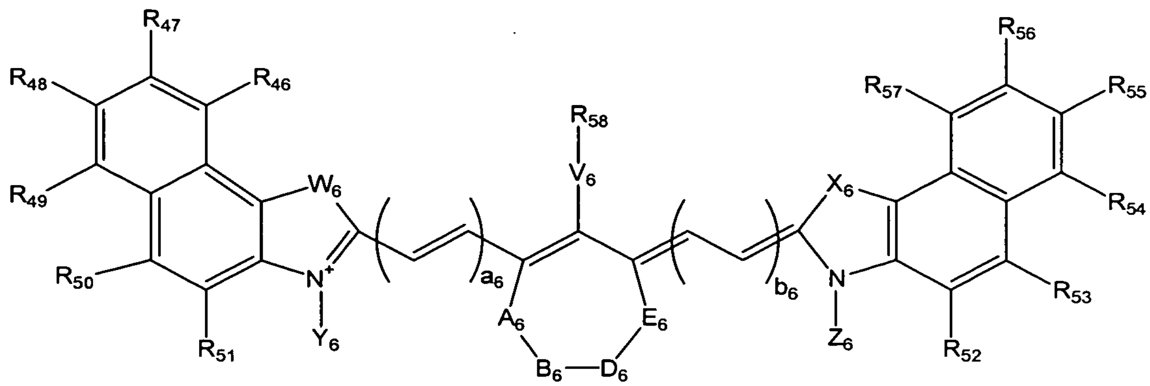


Formula 5

wherein R_{37}, R_{38}, R_{39}, R_{40}, R_{41}, R_{42}, R_{43}, R_{44}, R_{45}, Y_5, and Z_5 are independently selected from the group consisting of -H, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, C1-C20 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, saccharides, amino, C1-C10 aminoalkyl, cyano, nitro, halogen, hydrophilic peptides, arylpolysulfonates, C1-C10 alkyl, C1-C10 aryl, -SO_3T, -CO_2T, -OH, -$(CH_2)_aSO_3T$, -$(CH_2)_aOSO_3T$, -$(CH_2)_aNHSO_3T$, -$(CH_2)_aCO_2(CH_2)_bSO_3T$, -$(CH_2)_aOCO(CH_2)_bSO_3T$, -$(CH_2)_aCONH(CH_2)_bSO_3T$, -$(CH_2)_aNHCO(CH_2)_bSO_3T$, -$(CH_2)_aNHCONH(CH_2)_bSO_3T$,

$-(CH_2)_aNHCSNH(CH_2)_bSO_3T$, $-(CH_2)_aOCONH(CH_2)_bSO_3T$, $-(CH_2)_aPO_3HT$, $-(CH_2)_aPO_3T_2$, $-(CH_2)_aOPO_3HT$, $-(CH_2)_aOPO_3T_2$, $-(CH_2)_aNHPO_3HT$, $-(CH_2)_aNHPO_3T_2$, $-(CH_2)_aCO_2(CH_2)_bPO_3HT$, $-(CH_2)_aCO_2(CH_2)_bPO_3T_2$, $-(CH_2)_aOCO(CH_2)_bPO_3HT$, $-(CH_2)_aOCO(CH_2)_bPO_3T_2$, $-(CH_2)_aCONH(CH_2)_bPO_3HT$, $-(CH_2)_aCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCO(CH_2)_bPO_3HT$, $-(CH_2)_aNHCO(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCONH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3T_2$, $-(CH_2)_aOCONH(CH_2)_bPO_3HT$, and $-(CH_2)_aOCONH(CH_2)_bPO_3T_2$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_5 and X_5 are selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; V_5 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and $-NR_a$; D_5 is a single or a double bond; A_5, B_5 and E_5 may be the same or different and are selected from the group consisting of -O-, -S-, -Se-, -P-, $-NR_a$, $-CR_cR_d$, CR_c, alkyl, and -C=O; A_5, B_5, D_5, and E_5 may together form a 6 or 7 membered carbocyclic ring or a 6 or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or a sulfur atom; a, b, d, f, h, i, and j independently vary from 1-10; c, e, g, and k independently vary from 1-100; a_5 and b_5 vary from 0 to 5; R_a, R_b, R_c, and R_d are defined in the same manner as Y_5; T is either H or a negative charge.

The present invention also relates to the novel composition comprising cyanine dyes of general Formula 6



Formula 6

wherein R_{46}, R_{47}, R_{48}, R_{49}, R_{50}, R_{51}, R_{52}, R_{53}, R_{54}, R_{55}, R_{56}, R_{57} and R_{58}, Y_6, and Z_6 are independently selected from the group consisting of -H, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, C1-C20 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, saccharides, amino, C1-C10 aminoalkyl, cyano, nitro, halogen, hydrophilic peptides, arylpolysulfonates, C1-C10 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-(CH_2)_aCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCO(CH_2)_bSO_3T$, $-(CH_2)_aNHCONH(CH_2)_bSO_3T$, $-(CH_2)_aNHCSNH(CH_2)_bSO_3T$, $-(CH_2)_aOCONH(CH_2)_bSO_3T$, $-(CH_2)_aPO_3HT$, $-(CH_2)_aPO_3T_2$, $-(CH_2)_aOPO_3HT$, $-(CH_2)_aOPO_3T_2$, $-(CH_2)_aNHPO_3HT$, $-(CH_2)_aNHPO_3T_2$, $-(CH_2)_aCO_2(CH_2)_bPO_3HT$, $-(CH_2)_aCO_2(CH_2)_bPO_3T_2$, $-(CH_2)_aOCO(CH_2)_bPO_3HT$, $-(CH_2)_aOCO(CH_2)_bPO_3T_2$, $-(CH_2)_aCONH(CH_2)_bPO_3HT$, $-(CH_2)_aCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCO(CH_2)_bPO_3HT$, $-(CH_2)_aNHCO(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCONH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCONH(CH_2)_bPO_3T_2$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3HT$, $-(CH_2)_aNHCSNH(CH_2)_bPO_3T_2$, $-(CH_2)_aOCONH(CH_2)_bPO_3HT$, and $-(CH_2)_aOCONH(CH_2)_bPO_3T_2$, $-CH_2(CH_2$-O-

$CH_2)_c$-CH_2-OH, -$(CH_2)_d$-CO_2T, -CH_2-$(CH_2$-O-$CH_2)_e$-CH_2-CO_2T, -$(CH_2)_f$-NH_2, -CH_2-$(CH_2$-O-$CH_2)_g$-CH_2-NH_2, -$(CH_2)_h$-$N(R_a)$-$(CH_2)_i$-CO_2T, and -$(CH_2)_j$-$N(R_b)$-CH_2-$(CH_2$-O-$CH_2)_k$-CH_2-CO_2T; W_6 and X_6 are selected from the group consisting of -CR_cR_d, -O-, -NR_c, -S-, and -Se; V_6 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and -NR_a; D_6 is a single or a double bond; A_6, B_6 and E_6 may be the same or different and are selected from the group consisting of -O-, -S-, -Se-, -P-, -NR_a, -CR_cR_d, CR_c, alkyl, and -C=O; A_6, B_6, D_6, and E_6 may together form a 6 or 7 membered carbocyclic ring or a 6 or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or sulfur atom; a, b, d, f, h, i, and j independently vary from 1-10; c, e, g, and k independently vary from 1-100; a_6 and b_6 vary from 0 to 5; R_a, R_b, R_c, and R_d are defined in the same manner as Y_6; T is either H or a negative charge.

The inventive compositions and methods are advantageous since they provide a real-time, accurate, repeatable measure of renal excretion rate using exogenous markers under specific yet changing circumstances. This represents a substantial improvement over any currently available or widely practiced method, since currently, no reliable, continuous, repeatable bedside method for the assessment of specific renal function by optical methods exists. Moreover, since the inventive method depends solely on the renal elimination of the exogenous chemical entity, the measurement is absolute and requires no subjective interpretation based on age, muscle mass, blood pressure, etc. In fact it represents the nature of renal function in

this particular patient, under these particular circumstances, at this precise moment in time.

The inventive compounds and methods provide simple, efficient, and effective monitoring of organ function. The compound is administered and a sensor, either external or internal, is used to detect absorption and/or emission to determine the rate at which the compound is cleared from the blood. By altering the R groups, the compounds may be rendered more organ specific.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Reaction pathway for the preparation of indole derivatives.

Figure 2: Reaction pathway for the preparation of benzoindole derivatives.

Figure 3: Reaction pathway for the preparation of indocarbocyanine derivatives.

Figure 4: Reaction pathway for the preparation of benzoindocarbocyanine derivatives.

Figure 5: Reaction pathway for the preparation of robust indocarbocyanine derivatives.

Figure 6: Reaction pathway for the preparation of robust benzoindocarbocyanine derivatives.

Figure 7: Reaction pathway for the preparation of long-wavelength absorbing indocarbocyanine derivatives.

Figure 8a: Absorption spectrum of indoledisulfonate in water.

Figure 8b: Emission spectrum of indoledisulfonate in water.

Figure 9a: Absorption spectrum of indocarbocyaninetetrasulfonate in water.

Figure 9b: Emission spectrum of indocarbocyaninetetrasulfonate in water.

Figure 10a: Absorption spectrum of chloroindocarbocyanine in acetonitrile.

Figure 10b: Emission spectrum of chloroindocarbocyanine in acetonitrile.

Figure 11: Blood clearance profile of carbocyanine-polyaspartic (10 kDa) acid conjugate in a rat.

Figure 12: Blood clearance profile of carbocyanine-polyaspartic (30 kDa) acid conjugate in a rat.

Figure 13: Blood clearance profile of indoledisulfonate in a rat.

Figure 14: Blood clearance profile of carbocyaninetetrasulfonates in a rat.

DETAILED DESCRIPTION

In one embodiment of the invention, the dyes of the invention serve as probes for continuous monitoring of renal function, especially for critically ill patients and kidney transplant patients.

In another aspect of the invention, the dyes of the invention are useful for dynamic hepatic function monitoring, especially for critically ill patients and liver transplant patients.

In yet another aspect of the invention, the dyes of the invention are useful for real-time determination of cardiac function, especially in patients with cardiac diseases.

In still another aspect of the invention, the dyes of the invention are useful for monitoring organ perfusion, especially for critically ill, cancer, and organ transplant patients.

The novel dyes of the present invention are prepared according the methods well known in the art, as illustrated in general in Figures 1-7 and described for specific compounds in Examples 1-11.

In one embodiment, the novel compositions, also called tracers, of the present invention have the Formula 1, wherein R_3, R_4, R_5, R_6 and R_7, and Y_1 are independently selected from the group consisting of -H, C1-C5 alkoxyl, C1-C5 polyalkoxyalkyl, C1-C10 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, mono- and disaccharides, nitro, hydrophilic peptides, arylpolysulfonates, C1-C5 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_1 is selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; a, b, d, f, h, I, and j independently vary from 1-5; c, e, g, and k independently vary from 1-20; R_a, R_b, R_c, and R_d are defined in the same manner as Y_1; T is a negative charge.

In another embodiment, the novel compositions of the present invention have the general Formula 2, wherein R_8, R_9, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, and Y_2 are independently selected from the group consisting of -H, C1-C5 alkoxyl, C1-C5 polyalkoxyalkyl, C1-C10 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, mono- and disaccharides, nitro, hydrophilic peptides, arylpolysulfonates, C1-C5 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_2 is selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; a, b, d, f, h, I, and j independently vary from 1-5; c, e, g, and k independently vary from 1-20; R_a, R_b, R_c, and R_d are defined in the same manner as Y_2; T is a negative charge.

In another embodiment, the novel compositions of the present invention have the general Formula 3, wherein R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, Y_3, and Z_3 are independently selected from the group consisting of -H, C1-C5 alkoxyl, C1-C5 polyalkoxyalkyl, C1-C10 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, mono- and disaccharides, nitro, hydrophilic peptides, arylpolysulfonates, C1-C5 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_3 and

X_3 are selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; V_3 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and $-NR_a$; a, b, d, f, h, i, and j independently vary from 1-5; c, e, g, and k independently vary from 1-50; a_3 and b_3 vary from 0 to 5; R_a, R_b, R_c, and R_d are defined in the same manner as Y_3; T is either H or a negative charge.

In another embodiment, the novel compositions of the present invention have the general Formula 4, wherein R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, Y_4, and Z_4 are independently selected from the group consisting of -H, C1-C5 alkoxyl, C1-C5 polyalkoxyalkyl, C1-C10 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, mono- and disaccharides, nitro, hydrophilic peptides, arylpolysulfonates, C1-C5 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_4 and X_4 are selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; V_4 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and $-NR_a$; a_4 and b_4 vary from 0 to 5; a, b, d, f, h, i, and j independently vary from 1-5; c, e, g, and k independently vary from 1-50; R_a, R_b, R_c, and R_d are defined in the same manner as Y_4; T is either H or a negative charge.

In another embodiment, the novel compositions of the present invention have the general Formula 5, wherein R_{37}, R_{38}, R_{39}, R_{40}, R_{41}, R_{42}, R_{43}, R_{44}, R_{45}, Y_5, and Z_5 are independently selected from the group consisting

of -H, C1-C5 alkoxyl, C1-C5 polyalkoxyalkyl, C1-C10 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, mono- and disaccharides, nitro, hydrophilic peptides, arylpolysulfonates, C1-C5 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$, $-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_5 and X_5 are selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; V_5 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and $-NR_a$ D_5 is a single or a double bond; A_5, B_5 and E_5 may be the same or different and are selected from the group consisting of -O-, -S-, $-NR_a$, $-CR_cR_d$, CR_c, and alkyl; A_5, B_5, D_5, and E_5 may together form a 6 or 7 membered carbocyclic ring or a 6 or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or sulfur atom; a, b, d, f, h, i, and j independently vary from 1-5; c, e, g, and k independently vary from 1-50; a_5 and b_5 vary from 0 to 5; R_a, R_b, R_c, and R_d are defined in the same manner as Y_5; T is either H or a negative charge.

In yet another embodiment, the novel compositions of the present invention have the general Formula 6, wherein R_{46}, R_{47}, R_{48}, R_{49}, R_{50}, R_{51}, R_{52}, R_{53}, R_{54}, R_{55}, R_{56}, R_{57}, R_{58}, Y_6, and Z_6 are independently selected from the group consisting of -H, C1-C5 alkoxyl, C1-C5 polyalkoxyalkyl, C1-C10 polyhydroxyalkyl, C5-C20 polyhydroxyaryl, mono- and disaccharides, nitro, hydrophilic peptides, arylpolysulfonates, C1-C5 alkyl, C1-C10 aryl, $-SO_3T$, $-CO_2T$, -OH, $-(CH_2)_aSO_3T$, $-(CH_2)_aOSO_3T$, $-(CH_2)_aNHSO_3T$,

$-(CH_2)_aCO_2(CH_2)_bSO_3T$, $-(CH_2)_aOCO(CH_2)_bSO_3T$, $-CH_2(CH_2\text{-}O\text{-}CH_2)_c\text{-}CH_2\text{-}OH$, $-(CH_2)_d\text{-}CO_2T$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_e\text{-}CH_2\text{-}CO_2T$, $-(CH_2)_f\text{-}NH_2$, $-CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_g\text{-}CH_2\text{-}NH_2$, $-(CH_2)_h\text{-}N(R_a)\text{-}(CH_2)_i\text{-}CO_2T$, and $-(CH_2)_j\text{-}N(R_b)\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_k\text{-}CH_2\text{-}CO_2T$; W_6 and X_6 are selected from the group consisting of $-CR_cR_d$, -O-, $-NR_c$, -S-, and -Se; V_6 is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and $-NR_a$; D_6 is a single or a double bond; A_6, B_6 and E_6 may be the same or different and are selected from the group consisting of -O-, -S-, $-NR_a$, $-CR_cR_d$, CR_c, and alkyl; A_6, B_6, D_6, and E_6 may together form a 6 or 7 membered carbocyclic ring or a 6 or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or sulfur atom; a, b, d, f, h, i, and j independently vary from 1-5; c, e, g, and k independently vary from 1-50; a_5 and b_5 vary from 0 to 5; R_a, R_b, R_c, and R_d are defined in the same manner as Y_6; T is either H or a negative charge.

The dosage of the tracers may vary according to the clinical procedure contemplated and generally ranges from 1 picomolar to 100 millimolar. The tracers may be administered to the patient by any suitable method, including intravenous, intraperitoneal, or subcutaneous injection or infusion, oral administration, transdermal absorption through the skin, or by inhalation. The detection of the tracers is achieved by optical fluorescence, absorbance, or light scattering methods known in the art (Muller et al. Eds, Medical Optical Tomography, SPIE Volume IS11, 1993, which is expressly incorporated herein by reference) using invasive or non-invasive probes such as endoscopes, catheters, ear clips, hand bands, surface coils, finger probes, and the like. Physiological function is correlated with the clearance profiles

and rates of these agents from body fluids (R.B. Dorshow et al., Non-Invasive Fluorescence Detection of Hepatic and Renal Function, *Bull. Am. Phys. Soc.* 1997, 42, 681, which is expressly incorporated by reference herein).

The organ functions can be assessed either by the differences in the manner in which the normal and impaired cells remove the tracer from the bloodstream, by measuring the rate or accumulation of these tracers in the organs or tissues, or by obtaining tomographic images of the organs or tissues. Blood pool clearance may be measured non-invasively from convenient surface capillaries such as those found in an ear lobe or a finger, for example, using an ear clip or finger clip sensor, or may be measured invasively using an endovascular catheter. Accumulation of the tracer within the cells of interest can be assessed in a similar fashion. The clearance of the tracer dyes may be determined by selecting excitation wavelengths and filters for the emitted photons. The concentration-time curves may be analyzed in real time by a microprocessor. In order to demonstrate feasibility of the inventive compounds to monitor organ function, a non-invasive absorbance or fluorescence detection system to monitor the signal emanating from the vasculature infused with the compounds is used. Indole derivatives, such as those of Formulas 1-6, fluoresce at a wavelength between 350 nm and 1300 nm when excited at the appropriate wavelength as is known to, or readily determined by, one skilled in the art.

In addition to the noninvasive techniques, a modified pulmonary artery catheter can be used to make the necessary measurements (R.B. Dorshow, J.E. Bugaj, S.A. Achilefu, R. Rajagopalan, and A.H. Combs,

Monitoring Physiological Function by Detection of Exogenous Fluorescent Contrast Agents, in *Optical Diagnostics of Biological Fluids IV*, A. Priezzhev and T. Asakura, Editors, Procedings of SPIE 1999, 3599, 2-8, which is expressly incorporated by reference herein). Currently, pulmonary artery catheters measure only intravascular pressures, cardiac output and other derived measures of blood flow. Critically ill patients are managed using these parameters, but rely on intermittent blood sampling and testing for assessment of renal function. These laboratory parameters represent discontinuous data and are frequently misleading in many patient populations. Yet, importantly, they are relied upon heavily for patient assessment, treatment decisions, and drug dosing.

The modified pulmonary artery catheter incorporates an optical sensor into the tip of a standard pulmonary artery catheter. This wavelength specific optical sensor can monitor the renal function specific elimination of an optically detectable chemical entity. Thus, by a method analogous to a dye dilution curve, real-time renal function can be monitored by the disappearance of the optically detected compound. Modification of a standard pulmonary artery catheter only requires making the fiber optic sensor wavelength specific, as is known to one skilled in this art. Catheters that incorporate fiber optic technology for measuring mixed venous oxygen saturation currently exist.

The present invention may be used for rapid bedside evaluation of renal function and also to monitor the efficiency of hemodialysis. The invention is further demonstrated by the following examples. Since many

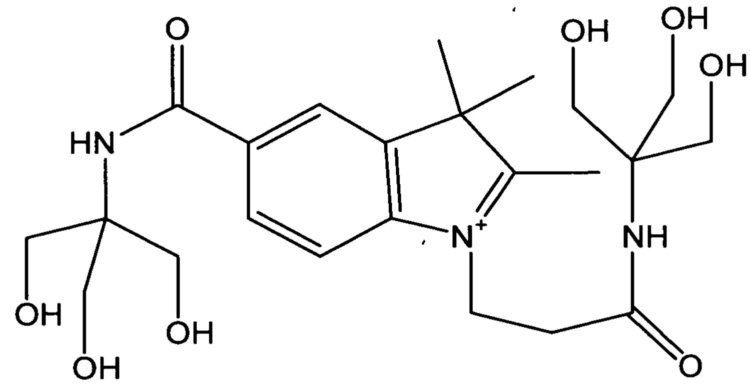
modifications, variations, and changes in detail may be made to the described embodiments, it is intended that all matter in the foregoing description and shown in the accompanying drawings be interpreted as illustrative and not in a limiting sense.

EXAMPLE 1

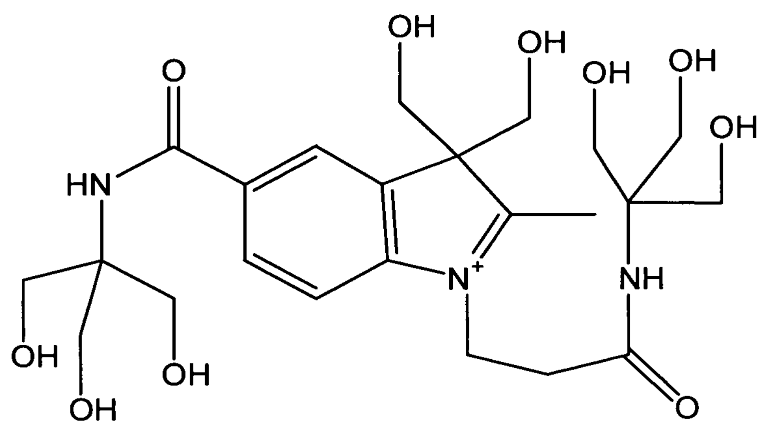
Synthesis of indole disulfonate
(Figure 1, Compound **5**, $Y_7 = SO_3^-$; $X_7 = H$; $n = 1$

A mixture of 3-methyl-2-butanone (25.2 mL), and p-hydrazinobenzenesulfonic acid (15 g) in acetic acid (45 mL) was heated at 110°C for 3 hours. After reaction, the mixture was allowed to cool to room temperature and ethyl acetate (100 mL) was added to precipitate the product, which was filtered and washed with ethyl acetate (100 mL). The intermediate compound, 2,3,3-trimethylindolenium-5-sulfonate (Figure 1, compound **3**) was obtained as a pink powder in 80% yield. A portion of compound **3** (9.2 g) in methanol (115 mL) was carefully added to a solution of KOH in isopropanol (100 mL). A yellow potassium salt of the sulfonate was obtained in 85% yield after vacuum-drying for 12 hours. A portion of the 2,3,3-trimethylindolenium-5-sulfonate potassium salt (4 g) and 1,3-propanesultone (2.1 g) was heated in dichlorobenzene (40 mL) at 110 °C for 12 hours. The mixture was allowed to cool to room temperature and the resulting precipitate was filtered and washed with isopropanol. The resulting pink powder was dried under vacuum to give 97% of the desired compound.

Other compounds prepared by a similar method described above include polyhydroxyl indoles such as



and



EXAMPLE 2

Synthesis of indole disulfonate (Figure 1, Compound **5**, $Y_7 = SO_3^-$; $X_7 = H$; $n = 2$)

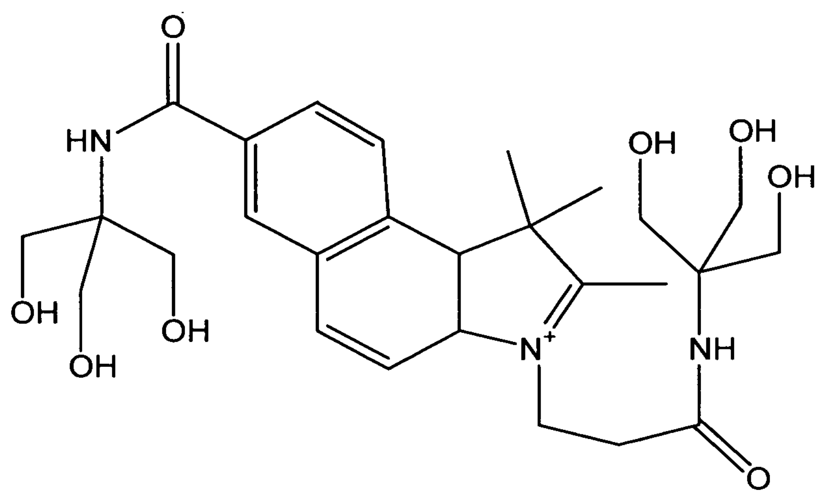
This compound was prepared by the same procedure described in Example 1, except that 1,4-butanesultone was used in place of 1,3-propanesultone.

EXAMPLE 3

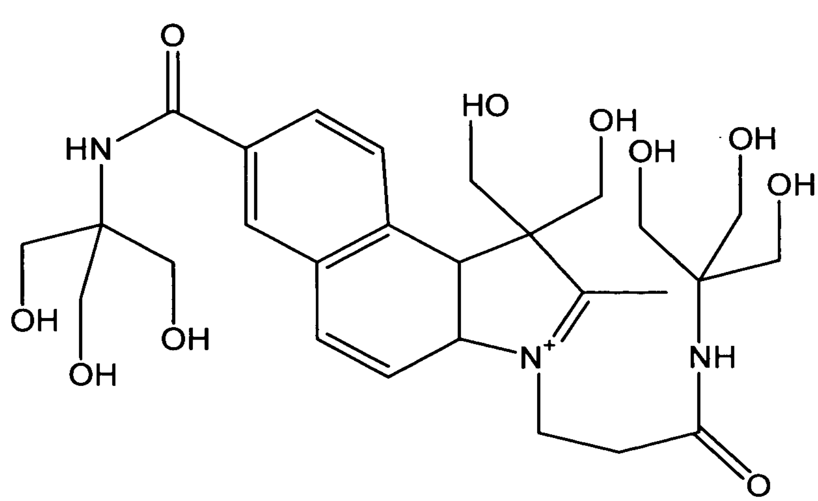
Synthesis of benzoindole disulfonate (Figure 2, Compound **8**, Y_7,Y_8 = SO_3^-; X_7 = H; n = 2)

This compound was prepared by the same procedure described in Example 1, except that hydrazinonaphthalenedisulfonic acid was used in place of hydrazinobenzenesulfonic acid.

Other compounds prepared by a similar method include polyhydroxyindoles such as:



and



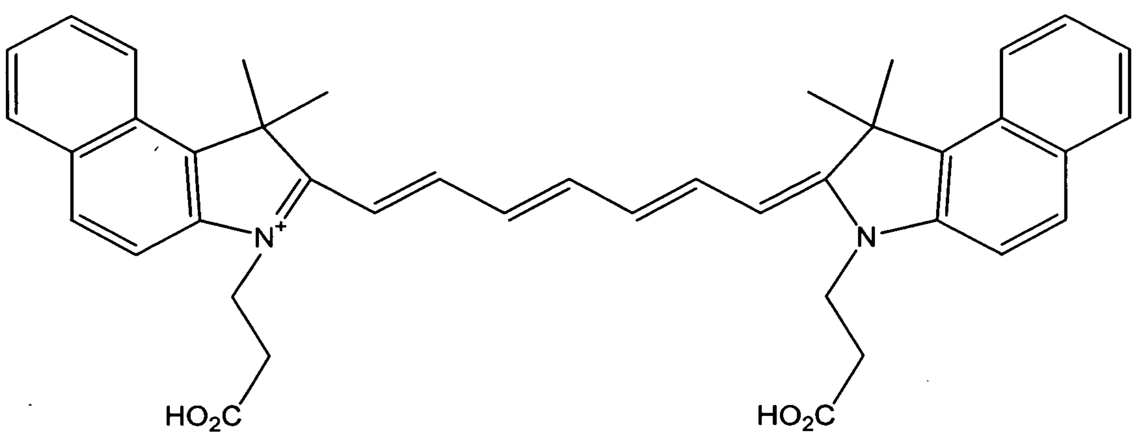
EXAMPLE 4

Synthesis of benzoindole disulfonate (Figure 2, Compound **8**, $Y_7, Y_8 = SO_3^-$; $X_7 = OH$; n = 4)

This compound was prepared by the same procedure described in Example 1, except that 3-hydroxymethyl-4-hydroxyl-2-butanone was used in place of 3-methyl-2-butanone.

EXAMPLE 5

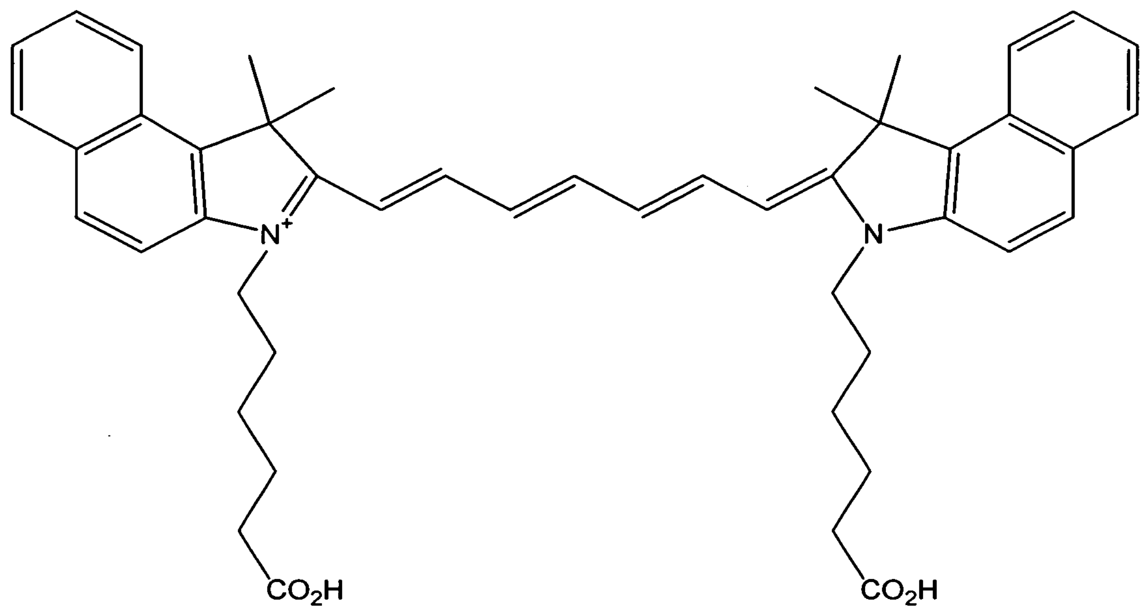
Synthesis of Bis(ethylcarboxymethyl)indocyanine Dye



A mixture of 1,1,2-trimethyl-[1H]-benz[e]indole (9.1 g, 43.58 mmoles) and 3-bromopropanoic acid (10.0 g, 65.37 mmoles) in 1,2-dichlorobenzene (40 mL) was heated at 110 °C for 12 hours. The solution was cooled to room temperature and the red residue obtained was filtered and washed with acetonitrile: diethyl ether (1:1) mixture. The solid obtained was dried under vacuum to give 10 g (64%) of light brown powder. A portion of this solid (6.0 g; 16.56 mmoles), glutaconaldehyde dianil monohydrochloride (2.36 g, 8.28 mmoles) and sodium acetate trihydrate (2.93 g, 21.53 mmoles) in ethanol (150 mL) were refluxed for 90 minutes. After evaporating the solvent, 40 mL of 2 N aqueous HCl was added to the residue and the mixture was centrifuged and the supernatant was decanted. This procedure was repeated until the supernatant became nearly colorless. About 5 mL of water:acetonitrile (3:2) mixture was added to the solid residue and lyophilized to obtain 2 g of dark green flakes. The purity of the compound was established with ^{1}H-NMR and liquid chromatography/mass spectrometry (LC/MS).

EXAMPLE 6

Synthesis of Bis(pentylcarboxymethyl)indocyanine Dye



A mixture of 2,2,3-trimethyl-[1H]-benz[e]indole (20 g, 95.6 mmoles) and 6-bromohexanoic acid (28.1 g, 144.1 mmoles) in 1,2-dichlorobenzene (250 mL) was heated at 110°C for 12 hours. The green solution was cooled to room temperature and the brown solid precipitate formed was collected by filtration. After washing the solid with 1,2-dichlorobenzene and diethyl ether, the brown powder obtained (24 g, 64%) was dried under vacuum at room temperature. A portion of this solid (4.0 g; 9.8 mmoles), glutaconaldehyde dianil monohydrochloride (1.4 g, 5 mmoles) and sodium acetate trihydrate (1.8 g, 12.9 mmoles) in ethanol (80 mL) were refluxed for 1 hour. After evaporating the solvent, 20 mL of a 2 N aqueous HCl was added to the residue and the mixture was centrifuged and the supernatant was decanted. This procedure was repeated until the

supernatant became nearly colorless. About 5 mL of water: acetonitrile (3:2) mixture was added to the solid residue and lyophilized to obtain about 2 g of dark green flakes. The purity of the compound was established with ^{1}H-NMR, HPLC, and LC-Mass spectrometry.

EXAMPLE 7

Synthesis of polyhydroxylindole sulfonate
(Figure 3, Compound **13**, Y_7,Y_8= O_3^-; X_7=OH; n=2)

Phosphorus oxychloride (37 ml, 0.4 mole) was added dropwise with stirring to a cooled (- 2 °C) mixture of dimethylformamide (DMF, 0.5 mole, 40 mL) and dichloromethane (DCM, 40 mL), followed by the addition of acetone (5.8 g, 0.1 mole). The ice bath was removed and the solution refluxed for 3 hours. After cooling to room temperature, the product was either partitioned in water/DCM, separated and dried, or was purified by fractional distillation. NMR and Mass spectral analyses showed that the desired intermediate, **10**, was obtained. Reaction of the intermediate with 2 equivalents of 2,2,3-trimethyl-[H]-benz[e]indolesulfonate-N-propanoic acid and 2 equivalents of sodium acetate trihydrate in ethanol gave a blue-green solution after 1.5 hours at reflux. Further functionalization of the dye with bis(isopropylidene)acetal protected monosaccharide is effected by the method described in the literature (J. H. Flanagan, C. V. Owens, S. E. Romero, et al., Near infrared heavy-atom-modified fluorescent dyes for base-calling in DNA-sequencing application using temporal discrimination. *Anal. Chem.*, 1998, 70(13), 2676-2684).

EXAMPLE 8

Synthesis of polyhydroxylindole sulfonate
(Figure 4, Compound **16**, Y_7,Y_8=SO_3^-; X_7=H; n=1)

Preparation of this compound is readily accomplished by the same procedure described in Example 6 using p-hydroxybenzenesulfonic acid in the place of the monosaccharide and benzoindole instead of indole derivatives.

EXAMPLE 9

Synthesis of polyhydroxylindole sulfonate
(Figure 5, Compound **20**, Y_7, Y_8=H; X_7=OH; n=1)

The hydroxy-indole compound is readily prepared by literature method (P. L. Southwick, J. G. Cairns, L. A. Ernst, and A. S. Waggoner, One pot Fischer synthesis of (2,3,3-trimethyl-3-H-indol-5-yl)-acetic acid derivatives as intermediates for fluorescent biolabels. *Org. Prep. Proced. Int. Briefs*, 1988, 20(3), 279-284). Reaction of p-carboxymethylphenylhydrazine hydrochloride (30 mmol, 1 equiv.) and 1,1-bis(hydroxymethyl)propanone (45 mmol, 1.5 equiv.) in acetic acid (50 mL) at room temperature for 30 minutes and at reflux for 1 gives (3,3-dihydroxymethyl2-methyl-3-H-indol-5-yl)-acetic acid as a solid residue.

The intermediate 2-chloro-1-formyl-3-hydroxymethylenecyclohexane was prepared as described in the literature (G. A. Reynolds and K. H. Drexhage, Stable heptamethine pyrylium dyes that absorb in the infrared. *J. Org. Chem.*, 1977, 42(5), 885-888). Equal volumes (40 mL each) of dimethylformamide (DMF) and dichloromethane were mixed

and the solution was cooled to -10°C in acetone-dry ice bath. Under argon atmosphere, phosphorus oxychloride (40 mL) in dichloromethane was added dropwise to the cool DMF solution, followed by the addition of 10 g of cyclohexanone. The resulting solution was allowed to warm up to room temperature and heated at reflux for 6 hours. After cooling to room temperature, the mixture was poured into ice-cold water and stored at 4°C for 12 hours. A yellow powder was obtained. Condensation of a portion of this cyclic dialdehyde (1 equivalent) with the indole intermediate (2 equivalents) was carried out as described in Example 5. Further the functionalization of the dye with bis (isopropylidene)acetal protected monosaccharide is effected by the method described in the literature (J. H. Flanagan, C. V. Owens, S. E. Romero, et al., Near infrared heavy-atom-modified fluorescent dyes for base-calling in DNA-sequencing application using temporal discrimination. *Anal. Chem.*, 1998, 70(13), 2676-2684).

EXAMPLE 10

Synthesis of polyhydroxylbenzoindole sulfonate
(Figure 6, Compound **22**, Y_7, Y_8=H; X_7=OH; n=1)

A similar method described in Example 8 is used to prepare this compound by replacing the indole with benzoindole derivatives.

EXAMPLE 11

Synthesis of rigid heteroatomic indole sulfonate (Figure 7, Compound **27**, Y_7, Y_8, X_7= H; n = 1)

Starting with 3-oxo-4-cyclohexenone, this heteroatomic hydrophilic dye is readily prepared as described in Example 8.

EXAMPLE 12

Minimally invasive monitoring of the blood clearance profile of the dyes

A laser of appropriate wavelength for excitation of the dye chromophore was directed into one end of a fiber optic bundle and the other end was positioned a few millimeters from the ear of a rat. A second fiber optic bundle was also positioned near the same ear to detect the emitted fluorescent light and the other end was directed into the optics and electronics for data collection. An interference filter (IF) in the collection optics train was used to select emitted fluorescent light of the appropriate wavelength for the dye chromophone.

Sprague-Dawley or Fischer 344 rats were used in these studies. The animals were anesthetized with urethane administered via intraperitoneal injection at a dose of 1.35 g/kg body weight. After the animals had achieved the desired plane of anesthesia, a 21 gauge butterfly with 12" tubing was placed in the lateral tail vein of each animal and flushed with heparinized saline. The animals were placed onto a heating pad and kept warm throughout the entire study. The lobe of the left ear was affixed to a glass microscope slide to reduce movement and vibration.

Incident laser light delivered from the fiber optic was centered on the affixed ear. Data acquisition was then initiated, and a background reading of fluorescence was obtained prior to administration of the test agent. The dyes were administered to the animal through a bolus injection in the lateral tail vein, typically 0.05 to 20 μmole.kg of body weight. The fluorescence signal rapidly increased to a peak value. The signal then decayed as a function of time as the conjugate cleared from the bloodstream.

This procedure was repeated with several dye-epetide conjugates in normal and tumored rats and representative profiles are shown in figures 6-10.

While the invention has been disclosed by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

What is claimed is: